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(54) Title: ADSORPTION MATRICES

(57) Abstract

New thiophilic adsorption matrices comprising a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, or thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover bound to a ligand, which is an aromatic or heteroaromatic ring system consisting of one or more rings, which are optionally substituted, but do not comprise nitrile groups; a process for producing them, and a process for purifying immunoglobulin from a liquid using thiophilic adsorption matrices.

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Adsorption Matrices

1. BACKGROUND OF THE INVENTION

The Technical Field

The invention concerns novel thiophilic adsorption matrices, preferably for use in the isolation, purification and immobilization of proteins from a liquid by salt-dependent adsorption chromatography, a process for producing them, and a process for purifying protein, preferably immunoglobulin.

Salt-dependent adsorption chromatography comprises binding proteins to an adsorption matrix in the presence of high concentrations of salts, in particular lyotropic (water-structure forming) salts. Specific binding of proteins is achieved by adjusting the salt concentration, and the bound proteins are eluted from the adsorption matrix by reducing the salt concentration in the medium.

Salt-dependent adsorption matrices are known, including e.g. metal chelate matrices, hydrophobic matrices and matrices of thiophilic nature. In the present specification the designation "thiophilic adsorption matrices" means hydrophilic adsorption matrices comprising a vinyl sulphone group to which a ligand is covalently bound, said matrices being capable of adsorbing proteins, such as serum proteins in the presence of high concentrations of lyotropic salts, such as alkali sulphates and alkali phosphates. This particularly distinct thiophilic adsorption nature is primarily believed to be caused by the sulphur atom in the divinyl sulphone group, to which should be added that the degree of thiophilic nature can be intensified or weakened by the ligand bound thereto.

Matrices exhibiting thiophilic adsorption are characterized by binding proteins in another manner than hydrophobic matrices. The differences are reflected i.a. in the fact that thiophilic matrices bind immunoglobulins from human serum much stronger than albumin at a given salt concentration. Albumin can be caused to bind to thiophilic matrices, but this typically requires a higher salt concentration than is necessary for binding immunoglobulin. Hydrophobic matrices are characterized by binding albumin more strongly than immunoglobulin.

Thiophilic adsorption matrices are used in particular in the fractionation of biopolymers, such as nucleic acids, nucleotides, and proteins, including serum proteins, immunoglobulins and enzymes, and other polypeptides.

Two types of adsorption matrices having a distinct thiophilic nature are known, the so-called T-gels and the nitrilophoric matrices. Furthermore, thio-aromatic matrices having a hydrophobic nature are known.

Prior Art

The US Patent Specification 4 696 980 describes thiophilic adsorption matrices which consist of hydrophilic matrices having covalently coupled chemical structures (ligands) of the type:

$$M\text{-}X\text{-}CH_2CH_2SO_2CH_2CH_2\text{-}S\text{-}Y,$$

wherein M is a polymeric hydrophilic network,

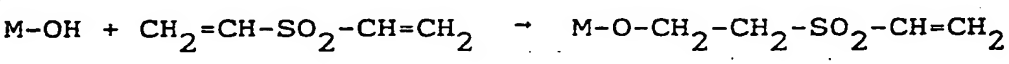
X is an O (oxygen), N (nitrogen) or S (sulphur) atom,

Y is an optionally substituted alkyl, aryl or heteroaromatic group, and

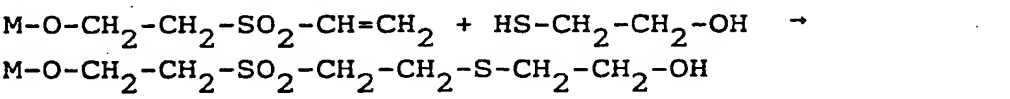
S is a sulphur atom positioned two carbon atoms away from the sulphone group and belonging to the ligand.

A typical matrix belonging to this group is produced by reaction of mercapto ethanol with a divinyl sulphone activated hydrophilic matrix (e.g. agarose):

First a hydrophilic matrix M-OH is activated with divinyl sulphone:



then the divinyl sulphone activated hydrophilic matrix is reacted with mercapto ethanol:



The thiophilic effect entails that these matrices do not bind albumin particularly effectively in contrast to known hydrophilic matrices which typically have long alkyl chains as ligands.

Thiophilic adsorption matrices of the above-mentioned type have surprisingly been found to have particular selectivity to immunoglobulins of the type IgG, IgA and IgM from serum and ascites liquids to which lyotropic salts, such as potassium sulphate have been added in a concentration of 0.5 M. After binding of immunoglobulin the matrix can be washed with an 0.5 M potassium sulphate buffer to remove non-bound contaminating proteins. The immunoglobulin can then be released by elution with a buffer with a low concentration of sodium chloride, e.g. 0.1 M sodium chloride.

This approach involves a rapid and convenient method of purifying immunoglobulins from liquids containing relatively high concentrations of immunoglobulin (above about 1 mg/ml), as is the case with e.g. serum and acites liquid.

However, thiophilic adsorption matrices of the above-mentioned type have a plurality of drawbacks associated with the exact chemical structure of the ligand.

As appears from the above chemical structure of thiophilic adsorption matrices, a sulphur atom is included, positioned at a distance of two carbon atoms after a sulphone group. In the past, this sulphur atom was considered essential to the quite special selectivity exhibited by the matrices, which appears from the EP Patent Specification 0 168 363 (example 4) and moreover from Jerker Porath and Makonnen Belew, Tibtech, 1987, vol. 5, p. 225-229, in particular page 226, where it is concluded that if the sulphur atom is replaced by nitrogen or oxygen the "thiophilic" nature will be much weaker, it being reported to decline through the series: S > N >> O,Se. This teaching suggests a strong restriction in the selection of possible and available substances that can be coupled to the divinyl sulphone activated matrix with the consequent desired effect. In addition, many thiol compounds, e.g. mercapto ethanol, are toxic and malodorous, and it is therefore strenuous to work with these because of the necessary safety measures which are to be taken in the coupling of the substance to the actived matrix. This applies in particular in case of a situation of use where the toxicity of the activation reagent, divinyl sulphone, has not been taken into account beforehand, which occurs e.g. if the activated matrix has been produced at an earlier time.

The US Patent Specification 3 897 467 describes nitrilophoric adsorbents comprising a hydrophilic polymer coupled with a ligand of the structure:

M-Y-X-R,

wherein R is an aliphatic or heteromatic substituent comprising at least one nitrile group,

X is NQ, S or O, wherein Q is $H(CH_2)_n$ with n = 0,1,2 or 3 and

Y is preferably $-CH_2-CH_2-SO_2-CH_2-CH_2-$

These adsorbents have properties which are comparable with the thiophilic matrices described above, but they do not necessarily comprise the essential sulphur atom two carbon atoms terminally from the sulphone group. Presumably, this can be ascribed to the presence of one or more nitrile groups in the ligand.

Nitrilophoric adsorbents seem to have a limited use, it being known that nitriles can hydrolyze in aqueous solutions, in particular in basic or acid solutions, which means that the adsorbents are unstable under conditions which are typically used for regeneration, sterilisation and depyrogenization (typically at a high or low pH and autoclaving) between and during use for purification of immunoglobulins.

The thiophilic and nitrilophoric adsorbents described above have been used for purification of immunoglobulin from a liquid according to a process comprising the following steps:

1) an adsorption step in which the liquid, from which immunoglobulin is to be purified, is admixed with a solution of 0.5 M potassium sulphate + 0.1 M Tris/HCl with pH 7.6, following which the liquid is contacted with the adsorbent, typically by passing the liquid through a column containing the adsorbent,

2) a washing step in which non-bound proteins are washed out of the adsorbent with a buffer solution containing 0.5 M potassium sulphate + 0.1 M Tris/HCl with pH 7.6, and

3) an elution step in which the bound proteins, including the immunoglobulins, are eluted from the adsorbent with a buffer solution having a low salt content, e.g. 0.05 M sodium phosphate with pH 8.0.

Hutchens and Porath, Analytical Biochemistry, vol. 159, p. 217-226, 1986 describe the process as a general and optimal method of purifying immunoglobulins from serum, and Belew, M et al., Journal of Immunological Methods, vol. 102, p. 173-182, 1987 describes such a process for purifying immunoglobulin from ascites liquid and in vitro cell culture supernatants containing monoclonal antibodies. As far as is known, no processes have been taught for purifying immunoglobulins by using different pH and ion strength during adsorption and washing phase. Lihme and Heegaard, Analytical Biochemistry, vol. 192, p. 64-69, 1991 describe the use of 0.75 M ammonium sulphate at neutral pH for purifying immunoglobulins from rabbit serum instead of 0.5 M potassium sulphate + 0.1 M Tris/HCl pH 7.6, as described above.

It is moreover known that other proteins than immunoglobulins, e.g. lentillectin and trypsin, Hutchens and Porath, Analytical Biochemistry, vol. 159, p, 217-226, 1986, and

serum albumin, Lihme and Heegaard, Analytical Biochemistry, vol. 192, p. 64-69, 1991 can be bound to thiophilic adsorbents if the ion strength is increased beyond what corresponds to 0.5 M potassium sulphate (I = 1.5). It is thus known that binding of serum albumin can take place at an ammonium sulphate concentration of about 1.2 M (I = 3.6), Lihme and Heegaard, Analytical Biochemistry, vol. 192, p. 64-69, 1991.

However, a drawback of the method is the provision of an eluate which, in addition to immunoglobulins, also contains significant amounts of contaminating proteins, such as e.g. transferrin and α-2-macroglobulin. Furthermore, the process has a relatively low capacity for binding of immunoglobulins from liquids having a particularly low concentration of immunoglobulin, e.g. in vitro cell culture supernatants which typically contain from 0.01 to 0.1 mg of immunoglobulin per ml.

These drawbacks limit the use of the separation principle and entail that it will often be necessary to combine the method with other supplementary purification steps, which both reduces the yield and adds to the costs.

2. DISCLOSURE OF THE INVENTION

The object of the present invention is to provide new alternative thiophilic adsorption matrices which are simple and inexpensive to produce, and which can be produced from divinyl sulphone activated matrices without special safety measures.

This object is achieved according to the invention by providing a thiophilic adsorption matrix comprising a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur

atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, characterized in that the ligand is a group of the general formula X-R, wherein X is NQ, wherein N is nitrogen, Q is $H(CH_2)_n$ and n = 0,1,2 or 3, or O (oxygen), and wherein R is an aromatic or heteroaromatic ring system which is optionally substituted, consisting of one or more rings, and R does not comprise nitrile groups.

It has surprisingly been found that the invention provides a thiophilic nature of the adsorption matrix which is at least equal to the thiophilic nature of known thiophilic matrices, if the ligand has an aromatic or heteroaromatic nature and the S coupling atom between the divinyl sulphone group and the ligand is replaced by O or N.

It is thus surprising that the reduced thiophilic nature of the adsorption matrix according to the prior art (Porath & Belew, ibid.) does not apply to the adsorption matrix of the invention.

Particularly preferred ligands are selected from the substituents stated in claim 2, the ligands stated in claim 3 being particularly preferred. In addition to being inexpensive, these ligand precursors are bound easily to the vinyl sulphone of a divinyl sulphone activated polymer network via oxygen or nitrogen belonging to the ligand. Furthermore, coupling of these precursors generally requires no special safety measures.

The ligand concentration may vary. Particularly preferred is a ligand concentration between 5 and 80 µmoles/ml, preferably between 5 and 40 µmoles/ml, especially between 10 and 40 µmoles/ml of wet matrix.

The hydrophilic network, to which the divinyl sulphone is bound, may either be natural or synthetic organic polymers respectively selected from: polysaccharides, such as agar, agarose, dextran, starch and cellulose, and synthetic organic polymers, such as polyacrylamide, polyamide, polyimide, polyester, polyether and polymeric vinyl compounds, and substituted derivatives thereof either as particles, membranes or contained in membranes. Agarose is particularly preferred.

In addition to being simple and inexpensive to produce, and without having to be produced under special safety measures, the novel thiophilic adsorption matrices of the invention are relatively stable in aqueous solutions under strongly acid or strongly basic conditions and at high temperatures.

Another object of the present invention is to provide a process for producing thiophilic adsorption matrices according to the invention. These thiophilic adsorption matrices comprise a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, said ligand being a group of the general formula X-R, wherein X is NQ, wherein N is nitrogen and Q is $H(CH_2)_n$ and n = 0,1,2,3 or O (oxygen), and wherein R is an aromatic or heteroaromatic ring system which is optionally substituted, consisting of one or more rings, and R does not comprise nitrile groups.

The polymer network is activated by being contacted with divinyl sulphone, following which the activated polymer network reacts with a ligand precursor. The ligand precursor is selected from the group consisting of 2-hydroxypyridine, 4-hydroxypyridine, xanthine, 4-methoxy-

phenol, 1-hydroxybenzotriazole, 4-aminobenzoic acid, 2-hydroxybenzylalcohol, 2,4-dihydroxy-6-methylpyridine, 4-aminosalicylic acid, 2-aminothiazole, 2-aminopyridine, 2-aminopyrimidine, 2-hydroxypyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4-triazole, 4-hydroxybenzoic acid butyl amide, 2-hydroxybenzhydroxamic acid, phenol and 4-chlorophenol.

Another object of the invention is to provide a process for purifying protein from a liquid.

This object is achieved by providing a process for purifying protein from a liquid, wherein the liquid is contacted with a thiophilic adsorption matrix, and the protein is then recovered from either the thiophilic adsorption matrix or from the liquid, characterized in that the thiophilic adsorption matrix is a thiophilic adsorption matrix according to the invention.

Possible proteins according to the invention are all proteins, in particular serum proteins, including immunoglobulins, albumin, α-1-antitrypsin, orosomucoid, Gc-globulin, and factor VIII, proteins from fermented liquids, including streptavidin and β-galactosidase, alkaline phosphatase from calf intestines, protein A and protein G.

Another object of the invention is to provide a process for purifying immunoglobulin from a liquid which gives a greater binding capacity than heretofore known methods by using both known thiophilic and nitrilophoric adsorption matrices and novel thiophilic matrices according to the invention. The object is particularly to provide a process for purifying immunoglobulins from a liquid with a concentration of than less than 1 mg of immunoglobulin per ml of liquid, including in vitro cell culture supernatants containing (murine) immunoglobulins of the type IgG_1, IgG_{2A},

IgG_{2B}, IgG_3, IgM and IgE.

This object is achieved by providing a process for purifying immunoglobulin, comprising adding a lyotropic buffer to the liquid, contacting the liquid with a thiophilic adsorption matrix, washing the thiophilic adsorption matrix with a lyotropic buffer solution, and eluting the washed thiophilic adsorption matrix with an elution liquid, characterized in that the lyotropic buffer in the liquid has an ion strength above 2.25, preferably between 2.25 and 4.5, in particular between 3.0 and 4.0.

It has surprisingly been found that an ion strength above 2.25 can result in a considerably greater capacity than the one achievable by known methods.

Another object of the invention is to provide a process giving a greater purity of the purified immunoglobulin than the known processes.

This object is achieved by providing a process for purifying immunoglobulin from a liquid, comprising adding a lyotropic buffer to the liquid, contacting the liquid with a thiophilic adsorption matrix, washing the thiophilic adsorption matrix with a lyotropic buffer solution, and eluting the washed thiophilic adsorption matrix with an elution liquid, characterized in that the lyotropic buffer solution has an ion strength below 2.25, preferably between 0 and 2.25, in particular between 0.6 and 1.5.

It has surprisingly been found that the low ion strength gives a greater purity of the eluted immunoglobulin, without this resulting in a smaller yield of the immunoglobulin.

In a particularly preferred embodiment pH in the lyotropic buffer solution is below 7.5, preferably between 2.5 and 7.5, in particular between 3.0 and 6.5, particularly preferred being 3.5 to 6.0, especially 4.0 to 5.5.

In another preferred embodiment the lyotropic buffer in the liquid has an ion strength above 2.25, preferably between 2.25 and 4.5, in particular between 3.0 and 4.0, thereby providing both a greater binding capacity and a greater purity than in the known methods.

Possible thiophilic adsorption matrices are known as well as novel thiophilic matrices of the invention. Thus, the thiophilic matrix is selected from divinyl sulphone activated polymer network, to which the divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, the divinyl sulphone groups being moreover covalently bound to a ligand selected from:

a) an alkyl, aryl or heteroaromatic group which is optionally substituted, and which is bound to a divinyl sulphone group via a sulphur atom,

b) an aromatic or heteroaromatic ring system, which is optionally substituted, consisting of one or more rings whose substituents do not comprise nitile groups, and which is bound to a divinyl sulphone group via an oxygen atom or a nitrogen atom, and

c) an aliphatic or a heterocyclic ring system having at least one nitrogen atom in the ring with one or more side groups consisting of or comprising nitrile groups, bound to a divinyl sulphone group via a sulphur atom, an oxygen atom or a nitrogen atom.

Moreover, a possible polymeric network for the known and novel thiophilic matrices of the invention is known polymer networks, such as polysaccharides, e.g. agar, agarose, dextran, starch and cellulose, in particular agarose, polyacrylamide, polyamide, polyimide, polyester, polyether, polymeric vinyl compounds and substituted derivatives thereof either as particles, membranes or contained in membranes.

Possible lyotropic salts for the lyotropic buffer and the lyotropic buffer solution according to the invention are known inorganic salts, such as sodium sulphate, potassium sulphate, ammonium sulphate, sodium phosphate, potassium phosphate and ammonium phosphate, or organic salts of polyvalent carboxylic acids, such as sodium citrate, sodium tartrate, potassium citrate, potassium tartrate, or mixtures thereof.

Possible liquids according to the invention are immunoglobulin-containing liquids, in particular biological liquids, such as blood, serum, ascites liquid or cell culture supernatants, in particular cell culture supernatants.

Possible immunoglobulins according to the invention are all immunoglobulins, in particular immunoglobulins of the type IgG_1, IgG_{2A}, IgG_{2B}, IgG_3, IgA, IgM, IgD and IgE and in particular murine and human immunoglobulins.

3. DETAILED DESCRIPTION

Thiophilic adsorption matrices

As mentioned, it has surprisingly been found that the replacement of the said sulphur atom by e.g. oxygen or nitrogen resulting in a reduced thiophilic efficiency

according to the prior art, unless Y contains one or more nitrile groups, does not apply if the ligand has an aromatic or heteroaromatic nature. In contrast, it has been found that matrices in which e.g. -S-R has been replaced by a phenyl ring, i.e. matrices having the structural formula:

$$M\text{-}X\text{-}CH_2CH_2SO_2CH_2CH_2\text{-}O\text{-}C_6H_5,$$

exhibit a particularly strong thiophilic binding, which is even stronger than the binding that can be achieved with the corresponding mercapto ethanol derivative according to the prior art. The characteristic thiophilic interaction of this phenyl derivatised matrix manifests itself in that human serum immunoglobulin G binds more strongly to the matrix than albumin. The stronger thiophilic binding of this matrix, compared with the mercapto ethanol derivative, manifests itself in that human immunoglobulin G is bound more effectively at lower concentrations of lyotropic salts in the sample. This is an effect which greatly depends upon the ligand concentration on the matrix, matrices with a high ligand concentration binding the immunoglobulin even in the absence of lyotropic salts, while matrices with a low or average ligand concentration require the presence of lyotropic salts to bind effectively. This stronger thiophilic binding of the immunoglobulin at high ligand concentrations may be desirable and advantageous in certain cases; e.g. in industrial applications where large amounts of liquid containing salts, such as ammonium sulphate or potassium sulphate, may pose a problem in terms of contamination.

It should moreover be noted that the thiophilic matrices are not only useful for binding and purifying immunoglobulins, but they can also be used for binding other proteins, such as other serum proteins than immunoglobulins,

depending upon the used concentration of lyotropic salts in the sample (Lihme & Heegaard, Analytical Biochemistry, vol. 192, p. 64-69, 1991). In this case too it will be advantageous with a smaller concentration of salts in the sample.

It is thus new and surprising that phenol coupled to divinyl sulphone activated agarose has a thiophilic nature in its protein adsorbing manner.

In a study of a plurality of different ligands containing an aromatic or heteroaromatic core with or without substituents, it has moreover been found that the sulphur atom can generally be replaced by oxygen or nitrogen while maintaining the thiophilic nature if Y has an aromatic or heteroaromatic nature. The following substances were coupled to divinyl sulphone activated agarose:

2-hydroxypyridine, 4-hydroxypyridine, xanthine, 4-methoxyphenol, 1-hydroxybenzotriazole, 4-aminobenzoic acid, 2-hydroxybenzylalcohol, 2,4-dihydroxy-6-methylpyrimidine, 4-aminosalicylic acid, 2-aminothiazole, 2-aminopyridine, 2-aminepyrimidine, 2-hydroxypyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4-triazole, 4-hydroxybenzoic acid butyl amide, 2-hydroxybenzhydroxamic acid, phenol and 4-chlorophenol. They all provided an adsorption matrix having a strong thiophilic nature.

It is common to the studied ligands that none of the resulting adsorption matrices bind albumin noticeably, while they all bind immunoglobulins of the type IgG, IgA and IgM extremely effectively and at least just as effectively as known adsorption matrices based on mercapto ethanol derivatives.

In addition to binding immunoglobulins strongly, it has been found that the various adsorption matrices exhibit small, but distinct differences in selectivity to binding of other proteins. For purification of specific proteins it is therefore important that the substitutents on the aromatic or heteroaromatic core are selected such as to provide the best possible capacity and purity. Thus, by routine tests a skilled person can select the ligand most suitable for a specific protein.

It has additionally also been found that various isomers of the same substance can exhibit great differences in binding strength as well as in selectivity. This is illustrated by coupling of 2-hydroxypyridine and 4-hydroxypyridine (examples 1 and 2), respectively, the 2-hydroxypyridine derivative binding significantly more protein than the 4-hydroxypyridine derivative. It is common to both derivatives that they do not bind albumin, or that the binding of albumin is very weak.

Purification of immunoglobulin

As mentioned, the known process for purifying immunoglobulins by means of thiophilic or nitrilophoric matrices is restricted i.a. because the binding capacity for immunoglobulin from liquids with very low concentrations of immunoglobulin (i.e. below about 1 mg/ml) is relatively low under known binding conditions (0.5 M potassium sulphate or 0.75 M ammonium sulphate). The low immunoglobulin concentration typically occurs with the cell culture supernatants resulting from the production of monoclonal antibodies by in vitro cultivation of hybridoma cells, in contrast to serum and ascites liquids which contain a much higher concentration of immunoglobulin.

However, it has surprisingly been found that the binding capacity for immunoglobulin to adsorption matrices having a thiophilic nature (including the known thiophilic and nitrilophoric as well as the new thiophilic matrices of the invention) increases steeply with increasing ion strength (concentration) of the known lyotropic salts in the liquid from which the immunoglobulin is to be purified.

It has thus been found that binding of monoclonal antibody from in vitro cell culture supernatants was strongly intensified by an increase of the ion strength in the liquid during the binding process, from 2.25 to 3.0 (corresponding to an increase in the ammonium sulphate concentration in the liquid from 0.75 M to 1 M). This increased binding strength results in a corresponding increase in the total binding capacity of 300-1000%. A comparison between 0.5 M potassium sulphate (ion strength = 1.5) and 1 M ammonium sulphate gave a correspondingly great increase in binding capacity. It should be noted that potassium sulphate has a maximum solubility of about 0.6 M and therefore cannot be brought up to the required ion strength.

An increase in the ion strength with ammonium sulphate to 3.8 and 4.0 gives an additional increase in binding strength, the rule being that the lower the immunoglobulin concentration, the higher the ion strength required to maintain the binding capacity. Accordingly, there is no upper limit to the ion strength of a lyotropic salt it is desirable to use.

However, depending upon the immunoglobulin concentration in the liquid, it will always be desirable to have a minimum ion strength of 2.4, and in typical cases with in vitro cell culture supernatants, with immunoglobulin concentrations between 0.01 mg/ml and 1 mg/ml, it is pre-

ferred to use ion strengths of between 2.7 and 4.5. For routine use, it is preferred to use ion strengths of between 3.0 and 4.0.

The salts capable of contributing to binding immunoglobulin to thiophilic and nitrilophoric adsorption matrices belong to the group of lyotropic (water structure forming) salts. Examples of such are salts containing sulphate or phosphate ions typically with sodium, potassium or ammonium ions as counter ions. Furthermore, some organic ions also have lyotropic activity, e.g. the multivalent anions of organic polyvalent carboxylic acids (e.g. citrate or tartrate ions). However, it has been found according to the invention that provision of an increased binding capacity by increasing the ion strength over the ion strength used in the prior art, is not limited to ammonium sulphate, but that the increased binding capacity achievable applies to all inorganic and organic salts having lyotropic properties.

The other mentioned drawback of known processes for purifying immunoglobulings by means of thiophilic or nitrilophoric adsorption matrices is that the eluted immunoglobulin (the product of the purification) is significantly contaminated by other proteins, e.g. transferrin and α-2-macroglobulin.

It has surprisingly been found that this contamination can greatly be prevented by flushing the adsorption matrix after the adsorption of the immunoglobulin with a solution of a lyotropic salt, which is present in an amount such that the ion strength in the solution is lower than the ion strength of the solution which was used for adsorbing the immunoglobulin to the adsorption matrix, and in certain cases that pH is below 7.5.

Hutchens and Porath, Analytical Biochemistry, vol. 159, p. 217-226, 1986 have described that binding of immunoglobulins to thiophilic adsorbents is pH dependent, but it has not previously been reported, disclosed or suggested that utilization of this pH dependency in connection with the flushing procedure after adsorption of the immunoglobulin should be able to increase the purity of the eluted immunoglobulin.

The process of the invention may e.g. be used in the purification of immunoglobulin from monoclonal in vitro cell culture supernatants according to the following specific procedure, comprising:

admixing the cell culture supernatant with ammonium sulphate and sodium acetate to a final concentration of 1.0 M and 0.05 M, respectively, pH 5.2, and then contacting the liquid with the adsorption matrix, typically by passing the liquid through a column containing it,

washing non-bound and bound contaminating proteins out of the matrix with a buffer solution consisting of 0.3 M ammonium sulphate + 0.05 M sodium acetate, pH 5.2, and

eluting the bound proteins, including immunoglobulins, with a buffer solution, which has a low salt content, e.g. 0.05 M Tris/HCl, pH 9.0.

The process of the invention may also be used for purifying immunoglobulins from other liquids, e.g. serum or ascites liquid, but then requires individual adjustment of the ion strength of the flushing buffer with respect to the lyotropic salt and the pH value of the flushing buffer. The result will be an increased purity of the immunoglobulin, without the binding capacity of the adsorption matrix being significantly diminished. Likewise,

individual adjustment of ion strength and pH value will be desirable depending upon the type of the immunoglobulin (for murine antibodies depending upon whether IgG_1, IgG_{2A}, IgG_{2B}, IgG_3, IgM or IgE is involved).

The preferred ion strength of the flushing buffer depends upon the specific application and the present contaminations, but is typically between 0 and 2.25. In most cases the most preferred range is between 0.6 and 1.5. In the same manner, the preferred pH value of the flushing buffer depends upon the specific application, but is typically between pH 2.5 and pH 7.5. Owing to the stability of the immunoglobulin during the flushing procedure and the efficiency of the process, a more preferred range will be between pH 3.0 and pH 6.5, while the most preferred range will be between pH 3.5 and pH 6.0. It is particularly preferred that pH is between 4.0 and 5.5.

The pH value of the elution buffer is preferably above 7.0, but may also be lower. Elution can also be performed by changing the dielectricity constant of the buffer, e.g. by addition of ethylene glycol. The selection of elution method is generally independent upon the process for binding the immunoglobulin and subsequent washing of contaminations.

4. EXAMPLES

Example 1

Production of 2-hydroxypyridine derivatised divinyl sulphone activated agarose

1) Divinyl sulphone activated agarose containing about 40 micromoles of vinyl sulphone groups per ml of wet drained gel (Mini Leak High, Kem-En-Tec, Denmark) was

washed on a suction filter with 2-3 volumes of ion exchanged water. The gel was drained by slight suction, and 10 g of wet drained gel were weighed.

2) 0.5 g of 2-hydroxypyridine was dissolved in 10 ml of 0.1 M dipotassium phosphate buffer, which was titrated to pH 11.0 with sodium hydroxide. After dissolution, the solution was again adjusted to pH 11.0. 2 mg of sodium boron hydride were added, and the resulting solution was mixed with the divinyl sulphone activated agarose. The gel was incubated with the solution for 18 hours at room temperature.

3) The gel was then washed thoroughly with 50% ethanol in water, with 25% ethanol in water and finally with pure water.

The resulting matrix contained about 40 micromoles of 2-hydroxypyrine per ml of wet drained matrix.

Example 2

Production of 4-hydroxypyridine derivatised divinyl sulphone activated agarose

The procedure was the same as described in example 1, but with 4-hydroxypyridine instead of 2-hydroxypyridine.

The resulting matrix contained about 40 micromoles of 4-hydroxypyrine per ml of wet drained matrix.

Example 3

Production of 4-methoxyphenol derivatised divinyl sulphone activated agarose

The procedure is the same as described in example 1, but with 4-methoxyphenol instead of 2-hydroxypyridine.

The resulting matrix contained about 40 micromoles of 4-methoxyphenol per ml of wet drained matrix.

Example 4

Production of 4-aminobenzoic acid derivatised divinyl sulphone activated agarose

The procedure was the same as described in example 1, but with 4-aminobenzoic acid instead of 2-hydroxypyridine.

The resulting matrix contained about 40 micromoles of 4-aminobenzoic acid per ml of wet drained matrix.

Example 5

Production of phenol derivatised divinyl sulphone activated agarose

The procedure was the same as described in example 1, but with phenol instead of 2-hydroxypyridine.

The resulting matrix contained about 40 micromoles of phenol per ml of wet drained matrix.

Example 6

Production of divinyl sulphone activated agarose coupled with various ligands

The procedure described in example 1 was used to produce divinyl sulphone activated agarose derivatized with the following ligand precursors:

xanthine, 1-hydroxybenzotriazole, 2-hydroxybenzylalcohol, 2,4-dihydroxy-6-methylpyrimidine, 4-aminosalicylic acid, 2-aminothiazole, 2-aminopyridine, 2-aminopyrimidine, 2-hydroxypyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4,-triazole, 2-hydroxybenzhydroxamic acid, and 4-chlorophenol

instead of 2-hydroxypyridine.

All compounds mentioned are commercially available.

The resulting matrices contained about 30-40 micromoles of ligands.

Example 7

Production of phenol derivatised divinyl sulphone activated agarose with medium ligand concentration

The procedure was the same as described in example 1, but with phenol instead of 2-hydroxypyridine, using a divinyl sulphone activated agarose containing 20 micromoles of vinyl sulphone groups per ml of wet matrix (Mini Leak Medium, Kem-En-Tec).

The resulting matrix contained about 20 micromoles of phenol per ml of wet drained matrix.

Example 8

Production of phenol derivatised divinyl sulphone activated agarose with low ligand concentration

The procedure was the same as described in example 1, but with phenol instead of 2-hydroxypyridine, using a divinyl sulphone activated agarose containing about 5 micromoles

of vinyl sulphone groups per ml of wet drained matrix (Mini Leak Low, Kem-En-Tec).

The resulting matrix contained about 5 micromoles of phenol per ml of wet drained matrix.

Example 9

Production of known mercapto ethanol derivatised divinyl sulphone activated agarose for comparison

1) Divinyl sulphone activated agarose containing about 40 micromoles of vinyl sulphone groups per ml of wet drained gel (Mini Leak High, Kem-En-Tec, Denmark) was washed on a suction filter with 2-3 volumes of ion exchanged water. The gel was drained by slight suction, and 10 g of wet drained gel were weighed.

2) 5% v/v mercapto ethanol in water was adjusted with sodium hydroxide to pH 9.5, and the resulting solution was mixed with a gel.

   The gel was incubated with the solution for 18 hours at room temperature.

3) The gel was then washed thoroughly with 50% ethanol in water, 25% ethanol in water and finally with pure water.

The resulting matrix contained about 40 micromoles of mercapto ethanol per ml of wet drained matrix.

Example 10

Prior art purification of immunoglobulins from human serum

The matrices, which were produced according to examples 1-4 and example 8, were used for purifying immunoglobulins from human serum according to the following known process (Lihme & Heegaard, Analytical Biochemistry, vol. 192, p. 64-69, 1991):

1) 3 ml of human serum were diluted 1:1 with 1.5 M $(NH_4)_2SO_4$.

2) The sample was applied to a column packed with 3 g of gel (respectively 2- and 4-hydroxypyridine, 4-methoxyphenol, 4-aminobenzoic acid and mercapto ethanol derivatised divinyl sulphone activated agarose). The respective columns have been equilibrated with 0.75 M $(NH_4)_2SO_4$ beforehand.

3) After application of the sample the gel was washed with 0.75 M $(NH_4)_2SO_4$ buffer until unbound proteins were washed out of the column.

4) The bound proteins, including the immunoglobulin, were eluted with 0.1 M NaCl.

As an expression of the bound and subsequently eluted amount of protein from the individual matrices, the EU figure of the eluate was calculated by means of the following formula:

(E_{280} of eluate) * (volume of eluate) = EU ("extinction units").

The qualitative composition of the eluates was determined by crossed immonoelectrophoresis. The result showed that:

$EU_{2\text{-hydroxypyridine}}$	= 15.7
$EU_{4\text{-hydroxypyridine}}$	= 11.4
$EU_{4\text{-methoxyphenol}}$	= 15.9
$EU_{4\text{-aminobenzoic acid}}$	= 13.8
$EU_{\text{mercapto ethanol}}$	= 15.3

The matrices of the invention and the known matrix (mercapto ethanol) thus bound comparable amounts of total protein and substantially the same amounts of immunoglobulin. None of the matrices bound albumin. The smaller amount of total protein eluted from the 4-hydroxypyridine matrix reflects a higher selectivity for immunoglobulin than the other matrices. It can therefore be concluded that the position of the substituent and on the whole the fine structure of the ligand have a decisive influence on the selectivity of the matrix.

Example 11

Known purification of proteins from human serum with phenol derivatised divinyl sulphone activated agarose matrices with different ligand concentration according to the invention

The influence of the ligand concentration on the protein fractionation was examined for the three phenol derivatised divinyl sulphone activated matrices, produced as described in examples 5, 7 and 8.

The raw material was human serum, and purification was performed so that the conditions during application promote binding of most serum proteins, (i.e. a higher ammonium sulphate concentration was used than the one used for

selective binding of immunoglobulins).

Process

1) Human serum was diluted 1:1 with 3.0 M $(NH_4)_2SO_4$ and centrifugated 30 minutes at 4000 rpm. The precipitate was discarded, while the supernatant was used for purification.

2) 1 ml of supernatant was then applied to 3 columns packed with 10 ml of phenol derivatised agarose containing about 40, about 20 and about 5 micromoles of phenol per ml of matrix (from examples 5, 7 and 8), respectively.

3) After application of the sample, the columns were flushed with 0.01 M K_2HPO_4, 1.5 M $(NH_4)_2SO_4$ buffer pH 7.2.

4) The matrices were eluted with a gradient from 0.01 M K_2HPO_4, 1.5 M $(NH_4)_2SO_4$ pH 7.2 to 0.01 M K_2HPO_4, 0.25 M NaCl pH 7.2.

The eluates were collected in fractions and analyzed by fused rocket immunoelectrophoresis for qualitative determination of the protein content.

The results showed that the adsorption matrix with the highest content of phenyl groups (40 micromoles/ml) bound the proteins most strongly, i.e. the proteins were generally eluted at a lower ion strength compared with the two other adsorption matrices. Furthermore, this matrix also bound the proteins more strongly than known thiophilic matrices, such as the mercapto ethanol derivative, the immunoglobulin G bound so strongly that it could only be liberated by a subsequent elution with 40% ethylene gly-

col. However, the thiophilic nature showed itself clearly in that albumin bound much more weakly to the matrix than immunoglobulin and much more weakly than the known hydrophobic matrices, such as octyl-Sepharose.

The adsorption matrix, with a content of about 20 micromoles of phenyl groups per ml of wet gel, exhibited a binding pattern which corresponds closely to the pattern achieved with known thiophilic matrices with a higher ligand concentration (about 40-60 micromoles/ml).

The adsorption matrix, with a content of about 5 micromoles of phenyl groups per ml, bound the proteins rather weakly, but still exhibited preference to binding of immunoglobulins.

All matrices bound immunoglobulin more strongly than albumin.

Example 12

Binding capacity for immunoglobulin from in vitro cell culture supernatant - comparison of processes

Process I (according to prior art)

1) 300 ml of in vitro hybridoma cell culture supernatant containing 50 µg of murine monoclonal immunoglobulin G_1 per ml and 10% v/v foetal calf serum are admixed with potassium sulphate and tris-hydroxymethyl aminomethane (Tris) to a final concentration of 0.5 M and 0.1 M, respectively, with pH adjusted with hydrochloric acid to 7.6.

2) The supernatant is passed through a column packed with 5 ml of 2-hydroxypyridine derivatised divinyl

sulphone activated agarose, as produced in example 1. The effluent from the column is collected in fractions, which are analyzed for their content of murine immunoglobulin by immunodiffusion.

Process II (according to the invention)

The test is performed like process I, but the potassium sulphate added in item 1 is replaced by 0.8 M ammonium sulphate, pH being kept constant at 7.6.

Process III

The test is performed like process II, the ammonium sulphate concentration being merely increased to 1.0 M.

Process IV

The test is performed like process II, the ammonium sulphate concentration being merely increased to 1.2 M.

The results are expressed as number of ml cell culture supernatant passing the column with the adsorption matrix before the effluent concentration of murine immunoglobulin is 50% of the start concentration (defined as "50% saturation").

Table I

	"50% saturation" ml
Process I	20
Process II	30
Process III	250
Process IV	>300

As will be seen from table I, increase in the ion strength of the medium results in a strong increase of the binding capacity for murine immunoglobulin.

Example 13

The test described in example 12 was repeated, the adsorption matrix being merely replaced by the mercapto ethanol derivative which was produced according to example 9.

Table I

	"50% saturation" ml
Process I	15
Process II	20
Process III	230
Process IV	>300

As will be seen, an increase in the ion strength also has a strong positive effect on the binding capacity of known thiophilic adsorption matrices.

Example 14

The test described in example 12 was repeated, the ammonium sulphate being merely replaced by sodium sulphate.

Table I

	"50% saturation" ml
Process I	30
Process II	40
Process III	>300
Process IV	>300

As will be seen, an increase in the ion strength also has a strong positive effect on the binding capacity when using sodium sulphate instead of ammonium sulphate.

Example 15

Purity of immunoglobulin isolated from in vitro cell culture supernatant - comparison of processes

Process I (known washing)

1) 300 ml of in vitro hybridoma cell culture supernatant containing 50 µg of murine monoclonal immunoglobulin G_1 per ml and 10% v/v foetal calf serum were admixed with ammonium sulphate and tris-hydroxymethyl aminomethane (Tris) to a final concentration of 1.0 M and 0.1 M, respectively, pH being adjusted with hydrochloric acid to 7.6.

2) The supernatant was passed through a column packed with 5 ml of 2-hydroxypyridine derivatised divinyl sulphone activated agarose, as produced according to example 1.

3) The column was washed with about 50 ml of 1.0 M ammonium sulphate + 0.01 M Tris/HCl, pH 7.6.

4) Bound proteins were released and eluted with 0.05 M Tris/HCl, pH 9.0.

The eluate was collected in one fraction and analyzed for purity by means of sodiumdodecyl polyacrylamide electrophoresis followed by electronic scanning. The yield in mg of murine immunoglobulin G_1 is determined by quantitative rocket immunoelectrophoresis. The purity is the immunoglobulin in % of the total amount of protein in the sample.

Process II

The test was performed like process I, the washing buffer in item 3 being merely replaced by 0.3 M ammonium sulphate + 0.05 M sodium acetate, pH 5.2.

Process III

The test was performed like process I, the washing buffer in item 3 being merely replaced by 0.3 M ammonium sulphate + 0.1 M Tris/HCl, pH 7.6.

	Yield, mg	Purity, %
Process I	11.5	about 30
Process II	11.0	- 90
Process III	<0.5	- 95

It will be seen from the results that a reduction in ion strength as well as pH in the flushing buffer increases the purity of the eluted immunoglobulin while retaining the high capacity. If pH is not reduced simultaneously with the ion strengh, a high purity is obtained, but the yield is very small.

Example 16

A comparative test of the process was performed like in example 15, the adsorption matrix being merely replaced by the mercapto ethanol derivative which was produced according to example 9.

The result corresponded completely to the result in example 15, which shows that the process of the invention also increases the purity of the eluted IgG for known thiophilic matrices.

Patent Claims:

1. A thiophilic adsorption matrix comprising a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, characterized in that the ligand is a group of the general formula X-R, wherein X is NQ, wherein N is nitrogen, Q is $H(CH_2)_n$ and n = 0,1,2 or 3, or O (oxygen), and wherein R is an aromatic or heteroaromatic ring system which is optionally substituted, consisting of one or more rings, and R does not comprise nitrile groups.

2. A thiophilic adsorption matrix according to claim 1, characterized in that R is substituted with substituents selected from:

alkyl, such as methyl, ethyl, propyl and butyl;
alkoxy, such as methoxy, ethoxy, propyloxy, butyloxy;
carboxyl, such as $-(CH_2)_nCOOH$, n = 0,1,2,3;
carboxamide, such as $-(CH_2)_nCONH_2$, n = 0,1,2,3;
carboxyhydroxyamide, such as $-(CH_2)_nCONHOH$, n = 0,1,2,3;
halogen, such as -F, -Cl, -Br and -I;
nitro, $-NO_2$;
sulphonic acid, $-SO_3H$;
hydroxyl, -OH;
alcohols, such as $-(CH_2)_nOH$, n = 1,2,3;
amines, such as $-(CH_2)_nNH_2$, n = 1,2,3.

3. A thiophilic adsorption matrix according to claims 1 and 2, characterized in that the ligand is coupled by reacting a ligand precursor selected from the group consisting of 2-hydroxypyridine, 4-hydroxypyridine, xanthine, 4-methoxyphenol, 1-hydroxybenzotriazole, 4-

aminobenzoic acid, 2-hydroxybenzylalcohol, 2,4-dihydroxy-6-methylpyrimidine, 4-aminosalicylic acid, 2-amino-thiazole, 2-aminopyridine, 2-aminopyrimidine, 2-hydroxy-pyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4-triazole, 4-hydroxybenzoic acid butyl amide, 2-hydroxy-benzhydroxamic acid, phenol and 4-chlorophenol with the divinyl sulphone groups of the hydrophilic polymer network.

4. A thiophilic adsorption matrix according to claims 1-3, c h a r a c t e r i z e d in that the ligand concentration is between 5 and 80 micromoles per ml of wet matrix, preferably between 5 and 40 micromoles per ml, in particular between 10 and 40 micromoles per ml of wet matrix.

5. A thiophilic adsorption matrix according to claims 1-4, c h a r a c t e r i z e d in that the polymeric network is selected from: polysaccharides, preferably agar, agarose, dextran, starch and cellulose, in particular agarose, and synthetic organic polymers, preferably polyacrylamide, polyamide, polyimide, polyester, polyether, polymeric vinyl compounds and substituted derivatives thereof either as particles, membranes or contained in membranes.

6. A process for producing a thiophilic adsorption matrix comprising a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, said ligand being a group of the general formula X-R, wherein X is NQ, wherein N is nitrogen and Q is $H(CH_2)_n$ and n = 0,1,2 or 3 or 0 (oxygen), and wherein R is an aromatic or heteroaromatic ring system which is optionally substituted, consisting of one or more rings, and R does

not comprise nitrile groups, wherein the polymer network is activated by contacting it with divinyl sulphone, and then the activated polymer network is reacted with a ligand precursor.

7. A process according to claim 6, c h a r a c t e r - i z e d in that the ligand precursor is selected from the following substances: 2-hydroxypyridine, 4-hydroxypyridine, xanthine, 4-methoxyphenol, 1-hydroxybenzotriazole, 4-aminobenzoic acid, 2-hydroxybenzylalcohol, 2,4-dihydroxy-6-methylpyrimidine, p-aminosalicylic acid, 2-aminothiazole, 2-aminopyridine, 2-aminopyrimidine, 2-hydroxypyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4-triazole, 4-hydroxybenzoic acid butyl amide, 2-hydroxybenzhydroxamic acid, phenol and 4-chlorophenol.

8. A process for purifying protein from a liquid, wherein the liquid is contacted with a thiophilic adsorption matrix, and the protein is then processed from either the thiophilic adsorption matrix or from the liquid, c h a r a c t e r i z e d in that the thiophilic adsorption matrix comprises a hydrophilic polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, said ligand being a group of the general formula X-R, wherein X is NQ, wherein N is nitrogen and Q is $H(CH_2)_n$ and n = 0-3, or 0 (oxygen), and wherein R is an aromatic or heteroaromatic ring system, which is optionally substituted, consisting of one or more rings, and R does not comprise nitrile groups.

9. A process according to claim 8, c h a r a c t e r - i z e d in that R is substituted with substituents selected from:

alkyl, such as methyl, ethyl, propyl and butyl;
alkoxy, such as methoxy, ethoxy, propyloxy, butyloxy;
carboxyl, such as $-(CH_2)_nCOOH$, n = 0,1,2,3;
carboxamide, such as $-(CH_2)_nCONH_2$, n = 0,1,2,3;
carboxyhydroxyamide, such as $-(CH_2)_nCONHOH$, n = 0,1,2,3;
halogen, such as -F, -Cl, -Br and -I;
nitro, $-NO_2$;
sulphonic acid, $-SO_3H$;
hydroxyl, -OH;
alcohols, such as $-(CH_2)_nOH$, n = 1,2,3;
amines, such as $-(CH_2)_nNH_2$, n = 1,2,3.

10. A process according to claims 8-9, c h a r a c - t e r i z e d in that the ligand is coupled by reacting a ligand precursor selected from the group consisting of 2-hydroxypyridine, 4-hydroxypyridine, xanthine, 4-methoxyphenol, 1-hydroxybenzotriazole, 4-aminobenzoic acid, 2-hydroxybenzyl alcohol, 2,4-dihydroxy-6-methylpyrimidine, 4-aminosalicylic acid, 2-aminothiazole, 2-aminopyridine, 2-aminopyrimidine, 2-hydroxypyrimidine, 4-hydroxypyrimidine, imidazole, 3-amino-1,2,4-triazole, 4-hydroxybenzoic acid butyl amide, 2-hydroxybenzoinhydroxamic acid, phenol or 4-chlorophenol with the divinyl sulphone groups of the hydrophilic polymer network.

11. A process for purifying immunoglobulin from a liquid, comprising adding a lyotropic buffer to the liquid, contacting the liquid with a thiophilic adsorption matrix, washing the thiophilic adsorption matrix with a lyotropic buffer solution, and then eluting the washed thiophilic adsorption matrix with an elution liquid, c h a r a c - t e r i z e d in that the lyotropic buffer in the liquid has an ion strength above 2.25, preferably between 2.25 and 4.5, in particular between 3.0 and 4.0.

12. A process for purifying immunoglobulin from a liquid, comprising adding a lyotropic buffer to the liquid, contacting the liquid with a thiophilic adsorption matrix, washing the thiophilic adsorption matrix with a lyotropic buffer solution, and then eluting the washed thiophilic adsorption matrix with an elution liquid, c h a r a c t e r i z e d in that the lyotropic buffer solution has an ion strength below 2.25, preferably between 0 and 2.25, in particular between 0.6 and 1.5.

13. A process according to claim 12, c h a r a c t e r i z e d in that pH of the lyotropic buffer solution is below 7.5, preferably between 2.5 and 7.5, in particular between 3.0 and 6.0, especially preferred being the range 3.5 to 6.0, in particular 4.0 to 5.5.

14. A process according to claims 12-13, c h a r a c t e r i z e d in that the lyotropic buffer in the liquid has an ion strength above 2.25, preferably between 2.25 and 4.5, in particular between 3.0 and 4.0.

15. A process according to claims 11-14, c h a r a c t e r i z e d in that the thiophilic adsorption matrix is selected from divinyl sulphone activated polymer network to which divinyl sulphone groups are bound via an ether oxygen atom, a thioether sulphur atom or a nitrogen atom, and the divinyl sulphone groups are moreover covalently bound to a ligand, which is selected from:

a) an alkyl, aryl or heteroaromatic group, which is optionally substituted and which is bound to a divinyl sulphone group via a sulphur atom,

b) an aromatic or heteroaromatic ring system, which is optionally substituted, consisting of one or more rings whose substituents do not comprise nitrile

groups, and which is bound to a divinyl sulphone via an oxygen atom or a nitrogen atom,

c) an aliphatic or a heterocyclic ring system having at least one nitrogen atom in the ring with one or more side groups consisting of or comprising nitrile groups bound to a divinyl sulphone group via a sulphur atom, an oxygen atom or a nitrogen atom.

16. A process according to claim 15, c h a r a c t e r i z e d in that the polymeric network is selected from polysaccharides, preferably agar, agarose, dextran, starch and cellulose, in particular agarose, and synthetic organic polymers, preferably polyacrylamide, polyamide, polyimide, polyester, polyether, polymeric vinyl compounds and substituted derivatives thereof either as particles, membranes or contained in membranes.

17. A process according to claims 11-16, c h a r a c t e r i z e d in that the lyotropic buffer and the lyotropic buffer solution comprise lyotropic salts selected from sodium sulphate, potassium sulphate, ammonium sulphate, sodium phosphate, potassium phosphate and ammonium phosphate, or organic salts of polyvalent carboxylic acids, such as sodium citrate, sodium tartrate, potassium citrate, potassium tartrate, or mixtures thereof.

18. A process according to claims 11-16, c h a r a c t e r i z e d in that the liquid is a biological liquid, preferably blood, serum, ascites liquid or cell culture supernatant, in particular cell culture supernatant.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 92/00092

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: B 01 J 20/22, 20/32, C 07 K 3/18, B 01 D 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	B 01 D; B 01 J; C 07 K; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	SE, B, 462165 (JERKER PORATH) 14 May 1990, see the whole document --	1-18
A	SE, B, 461505 (TANABE SEIYAKU CO LTD) 26 February 1990, see the whole document --	1-18
A	SE, B, 420733 (EXPLOATERINGS AB T.B.F.) 26 October 1981, see the whole document --	1-18
A	EP, A2, 0165912 (PORATH, JERKER) 27 December 1985, see the whole document --	1-18

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1st July 1992	1992 -07- 03
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Eva Iversen Hasselrot

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 92/00092

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 29/05/92
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